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WHAT'S KNOWN ON THIS SUBJECT: Moderate hypothermia showed some protection in selected groups of asphyxiated newborns. Combining hypothermia with postnatal pharmacologic treatment may further reduce post–hypoxic-ischemic reperfusion injury. Postnatal pharmacologic treatment, however, has not yet led to a significant improvement.

WHAT THIS STUDY ADDS: This is the first clinical study to investigate whether maternal treatment of the hypoxic fetus may lead to a reduction of the devastating consequences of birth asphyxia.

abstract

BACKGROUND: Fetal hypoxia is an important determinant of neonatal encephalopathy caused by birth asphyxia, in which hypoxia-induced free radical formation plays an important role.

HYPOTHESIS: Maternal treatment with allopurinol, will cross the placenta during fetal hypoxia (primary outcome) and reduce S-100B and free radical formation (secondary outcome).

METHODS: In a randomized, double-blind feasibility study, 53 pregnant women in labor (54 fetuses) with a gestational age of >36 weeks and fetal hypoxia, as indicated by abnormal/nonreassuring fetal heart rate tracing or fetal scalp pH of <7.20, received 500 mg of allopurinol or placebo intravenously. Severity of fetal hypoxia, brain damage and free radical formation were assessed by arterial cord blood lactate, S-100B and non-protein-bound-iron concentrations, respectively. At birth, maternal and cord blood concentrations of allopurinol and its active metabolite oxypurinol were determined.

RESULTS: Allopurinol and oxypurinol concentrations were within the therapeutic range in the mother (allopurinol > 2 mg/L and/or oxypurinol > 4 mg/L) but not always in arterial cord blood. We therefore created 3 groups: a placebo ($n = 27$), therapeutic allopurinol ($n = 15$), and subtherapeutic allopurinol group ($n = 12$). Cord lactate concentration did not differ, but S-100B was significantly lower in the therapeutic allopurinol group compared with the placebo and subtherapeutic allopurinol groups ($P < .01$). Fewer therapeutic allopurinol cord samples had measurable non–protein-bound iron concentrations compared with placebo ($P < .01$).

CONCLUSIONS: Maternal allopurinol/oxypurinol crosses the placenta during fetal hypoxia. In fetuses/newborns with therapeutic allopurinol/oxypurinol concentrations in cord blood, lower plasma levels of the brain injury marker protein S-100B were detected. A larger allopurinol trial in compromised fetuses at term seems warranted. The allopurinol dosage must be adjusted to achieve therapeutic fetal allopurinol/oxypurinol concentrations. *Pediatrics* 2009;124:350–357

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KEY WORDS
neuroprotection, allopurinol, fetal hypoxia, protein S-100B

ABBREVIATION
NPBI—non–protein-bound iron

This trial has been registered at www.clinicaltrials.gov (identifier NCT00189007).

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A more specific approach to improve neurodevelopmental outcome after perinatal hypoxia-ischemia or birth asphyxia is an important issue nowadays. Moderate hypothermia of the brain or the whole body has been proven to reduce brain damage after moderate-to-severe birth asphyxia.[1–4] However, it is conceivable that combining this technique with a pharmacologic means of neuroprotection after birth asphyxia will further improve neurodevelopmental outcome.[5] Experimental and clinical studies point to several compounds that may be good candidates for this purpose.[6–9] However, a major drawback in post-asphyxial hypothermia or pharmacologic treatment is the small therapeutic window (at best within 6 hours after birth) in which treatment should be initiated.[5,10] This window is even smaller when one aims to prevent or reduce free radical stress, an important cause of early reperfusion/reoxygenation injury to the immature brain. Free radical formation occurs on reperfusion and reoxygenation with its maximal formation within the first 30 minutes after birth.[11,12] The optimal point in time to start antioxidative treatment is at birth or even before birth. Because fetal hypoxia is an important determinant in the etiology of birth asphyxia-related encephalopathy, maternal treatment with antioxidative drugs, whether combined with early postnatal treatment such as hypothermia, may be a more optimal approach for reduction of reperfusion-reoxygenation injury after perinatal hypoxia-ischemia.

In the present feasibility study, we investigated whether allopurinol, a xanthine oxidase inhibitor, and in higher concentrations also a direct scavenger of the toxic hydroxyl free radical and a chelator of non–protein-bound iron (NPBI),[13–15] reduced free radical formation in the fetus. There were several reasons for choosing allopurinol: the increasing evidence that allopurinol and its active metabolite oxypurinol improve neurodevelopmental outcome after moderate-to-severe perinatal hypoxia-ischemia when used in the early neonatal period[9,16,17]; its proven ability to cross the human placenta[18]; and its proven safety profile.[9,13,16,18,19]

Therefore, we treated pregnant women with signs of fetal hypoxia, on the brink of delivery, in a double-blind, randomized manner, with intravenously administered allopurinol or a placebo. We hypothesized that maternally administered allopurinol crosses the placenta during fetal hypoxia (primary outcome), reduces fetal free radical formation, and ameliorates hypoxia-ischemia–related brain damage on the short-term as indicated by a decrease in fetal S-100B (secondary outcomes).

PATIENTS AND METHODS

Women in labor (gestational age of >36 weeks) with signs of fetal distress (as indicated by abnormal or nonreassuring fetal heart rate tracing as defined by the FIGO (International Federation of Gynecology and Obstetrics) criteria[20] or fetal scalp pH of <7.20[21]) were treated in a double-blind, randomized manner with either a single intravenous dose of 500 mg of allopurinol or a placebo (saline). Randomization was performed by the Department of Pharmacy of the University Medical Center Utrecht. Randomization sequence was generated by a computer program (Design 2.0 [Systat, Inc, Evanston, IL]) in blocks of 4. Patients were therefore randomly assigned to treatment with allopurinol or placebo according to this randomization sequence. Study drugs were prepared and labeled in a blind manner. Every site was supplied with study drug packages that were labeled with a unique set of numbers. The study drugs were stored at −20°C and thawed on the ward immediately before administration. Study medication was administered intravenously over 10 minutes after a thorough explanation and written consent from the mother and father, while the mother was being prepared for an emergency cesarean section or assisted vaginal delivery. In addition, women received acute tocolysis (either ritodrine or atosiban) to abolish additional detrimental effects of contractions on the fetal condition. The dose of allopurinol was derived from a study performed in healthy women in labor without fetal distress.[18] Women with signs of severe fetal distress who were being prepared for an emergency cesarean section were excluded from the study because of lack of time to properly prepare and perform the study protocol.

Maternal blood samples were obtained at the time of birth. Fetal blood samples were obtained from arterial cord blood. Maternal and cord blood investigation included plasma concentrations of allopurinol and oxypurinol and plasma concentrations of the free radical markers isoprostanes, total hydroperoxide, thiol groups, and NPBI. In addition, to assess the severity of fetal hypoxia, arterial cord lactate concentration was measured.[22] Protein S-100B concentration in arterial cord blood was used as a marker of central nervous system damage.[23–25] In addition, arterial cord plasma concentrations of liver functions (alanine aminotransferase, aspartate aminotransferase), renal function (urea, creatinine), uric acid, and troponin 1 (myocardial function marker) were determined. Blood samples for allopurinol and oxypurinol determination were collected in heparinized tubes, centrifuged, and stored at −20°C until analysis. Blood samples for evaluation

of oxidative stress were centrifuged at 2000 rpm for 10 minutes, and the supernatant was stored with butylated hydroxytoluene, to prevent continuation of the oxidation process, at a temperature of −80°C.

All of the neonates participating in the study were examined medically after birth. Adverse effects of allopurinol on the white blood cell count and on the skin were monitored in the mothers and children.[13,26]

The study was approved by the scientific boards and ethical committees of the participating hospitals (University Medical Center Utrecht, University Medical Center Groningen, and Universidad Del Norte, Barranquilla). Recruitment took place in 2006 and 2007.

Analysis of Allopurinol, Oxypurinol, Total Hydroperoxide, Thiol Groups, Isoprostanes, and NPBI

The allopurinol and oxypurinol plasma concentrations were determined by using reversed-phase, high-performance liquid chromatography with UV-detection at 254 nm for the quantification of allopurinol and oxypurinol in plasma.[27] The method was linear between 0.5 and 25 mg/L with a lower limit of quantification of 0.2 mg/L for both compounds. F2-isoprostanes in plasma were measured by using the method of Morrow,[28] by selected ion monitoring gas chromatography/negative ion chemical ionization-mass spectrometry employing [2H4] 8-iso-prostaglandin F2a as an internal standard. Total hydroperoxide production was measured with a d-ROMs test (Diacron International, Grosseto, Italy). Thiol production was measured with a SHp test (Diacron International), which is based on the ability of thiol groups to develop a photometrically detectable colored complex. The intensity of photometrically detected color is directly proportional to the concentration of thiols. Finally, detection of NPBI in plasma is based on preferential chelation of NPBI by a large excess of nitrilotriacetic acid, low affinity ligand (NTA). NTA captures all iron bound to low molecular weight proteins and nonspecifically bound to serum proteins; however, it does not remove iron bound to transferrin or ferritin.[29]

Statistical Analysis

Data are summarized as means ± SD or as median and ranges (shown as box-whisker plots [representing means, quartiles, and outliers]) where appropriate. Differences between the 2 groups were compared by the (unpaired) Student's t test, Mann-Whitney U test, or χ^2 test where appropriate. Differences between groups were assessed with one-factorial analysis of variance followed by the Scheffe procedure if a significant difference was found. A possible correlation was investigated with simple regression analysis. For statistical analysis, STAT VIEW II (Abacus Concepts, Inc, Berkeley, CA) was used. Statistical significance was set at $P < .05$.

RESULTS

Initially, 112 women were assessed for eligibility; 21 women refused to participate, 13 did not meet the inclusion criteria, and 25 were excluded for other reasons (such as insufficient time to properly prepare and perform the study protocol). A total of 53 mothers and 54 infants were included in the present study (Utrecht, $n = 38$; Groningen, $n = 4$; Barranquilla, $n = 11$): 27 placebo-treated mothers and 26 allopurinol-treated mothers (1 multiple gestation). The allopurinol group was subdivided into a therapeutic allopurinol and/or oxypurinol ($n = 15$) and a subtherapeutic allopurinol and/or oxypurinol ($n = 12$) group on the basis of cord concentrations of allopurinol and/or oxypurinol (see also below). Table 1 gives relevant maternal characteristics as a function of treatment. No differences were found for any of the parameters shown.

Table 2 provides relevant perinatal data of the neonates as a function of treatment. Gestational age, birth weight, Apgar score, arterial umbilical cord pH, and base excess did not differ between allopurinol- and placebo-treated newborns. Seventeen placebo, 6 subtherapeutic allopurinol and/or oxypurinol, and 5 therapeutic allopurinol and/or oxypurinol newborns had to be admitted to the neonatal ward; this was related to preceding fetal hypoxia in 8, 4, and 4 newborns, respectively. Duration of admittance ranged from 3 to 16 days for placebo-treated infants (median stay: 7 days) and from 1 to 20 days for allopurinol-treated infants (median stay: 8 days).

Table 3 summarizes chemical markers with respect to important organ systems such as the liver, renal function, and heart. No differences were detected between allopurinol- and placebo-treated groups. Troponin was not elevated in any of the therapeutic allopurinol and/or oxypurinol infants compared with 2 and 4 infants in the subtherapeutic allopurinol and/or oxypurinol and placebo groups, respectively.

Maternal and Arterial Cord Allopurinol and Oxypurinol Concentrations at Birth

The time from the start of maternal administration of allopurinol or placebo to birth ranged from 18 to 190 minutes (median: 56 minutes) and 12 to 372 minutes (median: 48 minutes), respectively. Maternal allopurinol and oxypurinol concentrations were significantly higher compared with arterial cord concentrations. Figure 1 shows the box-whisker plots of maternal and arterial cord concentrations. Maternal allopurinol and oxypurinol concentrations ranged between 1.2 and 7.9 μg/mL (median: 3.8 μg/mL) and be-

TABLE 1 Maternal Characteristics as a Function of Treatment Group

	Allopurinol and/or Oxypurinol		Placebo ($N = 27$)
	Therapeutic ($N = 14$)	Subtherapeutic ($N = 12$)	
Nulliparity, *n*	7	6	13
Maternal age, median (range), y	30 (26–33)	30 (22–43)	33.5 (24–42)
Multiple gestation, *n*	1	0	0
Time between infusion and delivery, median (range), min	56 (24–190)	48 (18–94)	41 (12–449)
Instrumented vaginal delivery, *n*	2	3	8
Spontaneous vaginal delivery, *n*	5	0	11
Cesarean delivery, *n*	7	9	8

TABLE 2 Perinatal and Neonatal Data as a Function of Treatment Group

	Allopurinol and/or Oxypurinol		Placebo ($N = 27$)
	Therapeutic ($N = 15$)	Subtherapeutic ($N = 12$)	
Gestational age, wk	40 ± 1	40 ± 1	40 ± 1
Birth weight, g	3148 ± 351	3194 ± 740	3189 ± 584
Male gender, *n*	8	8	15
Apgar score at 5 min	8.7 ± 1.3	9.3 ± 0.6	9.1 ± 0.8
Umbilical artery pH	7.17 ± 0.06	7.15 ± 0.02	7.14 ± 0.10
Umbilical artery BE	−10.3 ± 3.3	−8.3 ± 3.1	−8.9 ± 4.3

Values shown are mean ± SD or *n*. BE indicates base excess.

TABLE 3 Important Chemical Markers From the Arterial Cord Blood for Neonatal Liver, Kidney, Heart, and Brain as a Function of Treatment Group

	Allopurinol and/or Oxypurinol		Placebo ($N = 27$)
	Therapeutic ($N = 15$)	Subtherapeutic ($N = 12$)	
AST, U/L	43 (28–191)	40 (28–101)	44 (26–100)
ALT, U/L	16 (7–21)	19 (17–26)	17 (7–42)
LD, U/L	934 (541–5668)	1198 (439–4167)	1145 (352–1569)
Creatinine, μmol/L	73 (51–126)	68 (60–83)	67 (42–106)
Urea, mmol/L	3.4 (2.7–6.7)	3.4 (1.4–4.5)	3.4 (1.8–7.3)
Uric acid, mmol/L	0.36 (0.22–0.57)	0.30 (0.18–0.38)	0.32 (0.20–0.49)
Troponin, μg/L	0 (0–0)	0 (0–0.12)	0 (0–0.28)

Values shown are median (range). AST indicates aspartate aminotransferase; ALT, alanine aminotransferase; LD, lactate dehydrogenase.

tween 1.5 and 6.4 μg/mL (median: 3.2 μg/mL), respectively. The arterial cord allopurinol and oxypurinol concentrations ranged between 0.2 and 7.3 μg/mL (median: 2.0 μg/mL) and 0.6 and 7.6 μg/mL (median: 1.5 μg/mL), respectively. Both allopurinol and oxypurinol inhibit xanthine oxidase activity. On the basis of earlier (pharmacologic) studies, we considered allopurinol cord concentrations of ≥2.0 μg/mL and oxypurinol concentrations of ≥4.0 μg/mL to be in the therapeutic range in terms of xanthine oxidase inhibition.[30–32] This was the case in 15 of the allopurinol-treated newborns. The 12 remaining allopurinol-treated newborns had subtherapeutic levels. Oxypurinol, but not allopurinol, concentration in cord blood showed a positive correlation with time after maternal allopurinol administration ($r = 0.71$; $P < .001$).

Arterial Cord Lactate and S-100B Concentrations

Plasma lactate did not differ between groups. Figure 2 shows the box-whisker plots for plasma lactate. Plasma S-100B concentrations, however, were significantly lower in the therapeutic allopurinol and/or oxypurinol group, suggesting less brain damage compared with the placebo group. Figure 3A shows the box-whisker plots for the respective groups. Moreover, Figure 3B shows a significant correlation between the sum of allopurinol and oxypurinol concentrations on the one hand and S-100B on the other hand ($r = 0.59$; $P < .001$).

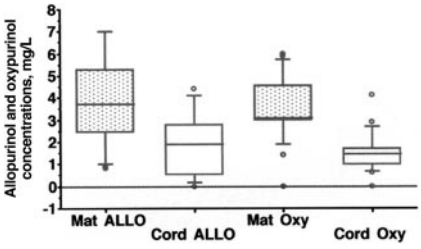

FIGURE 1
Maternal allopurinol (Mat ALLO) and oxypurinol (Mat Oxy), and arterial cord allopurinol (Cord ALLO) and oxypurinol (Cord Oxy) concentrations at birth shown as box-whisker plots.

Free Radical Markers in Maternal and Arterial Cord Plasma

Maternal free radical markers were always lower compared with arterial umbilical samples, except for total hydroperoxide concentrations. No significant differences were detected between the 3 groups with regard to isoprostane, thiol groups, total hy-

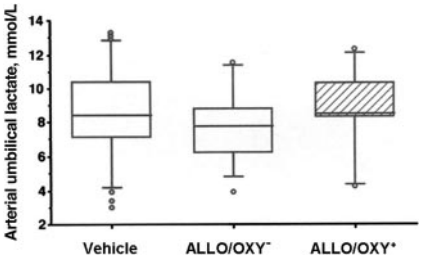

FIGURE 2
Arterial cord concentrations of lactate of the placebo-treated (placebo), therapeutic allopurinol and/or oxypurinol (ALLO/OXY$^+$), and subtherapeutic allopurinol and/or oxypurinol (ALLO/OXY$^-$) groups shown as box-whisker plots.

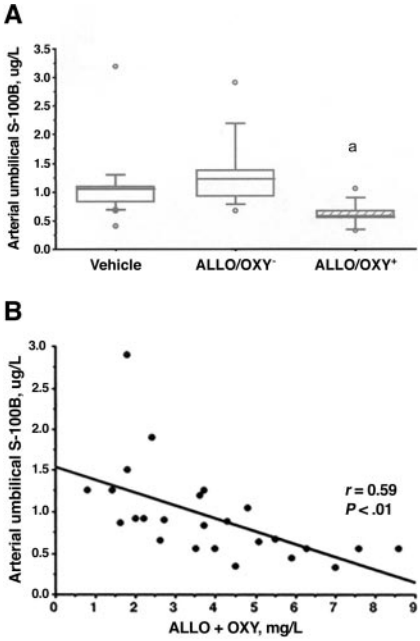

FIGURE 3

A, Arterial cord concentrations of S-100B (μg/L) of the placebo-treated (vehicle), therapeutic allopurinol and/or oxypurinol (ALLO/OXY$^+$), and subtherapeutic allopurinol and/or oxypurinol (ALLO/OXY$^-$) groups shown as box-whisker plots. [a] $P < .01$ versus placebo and subtherapeutic allopurinol and/or oxypurinol. B, Simple linear regression analysis between the sum of arterial cord concentrations of allopurinol and oxypurinol (ALLO$^+$OXY) as a function of S-100B.

droperoxide, or NPBI, although the latter 3 markers tended to be lower in the therapeutic allopurinol and/or oxypurinol group compared with the placebo group. Furthermore, NPBI was found significantly more in placebo and subtherapeutic allopurinol and/or oxypurinol cord blood compared with therapeutic allopurinol and/or oxypurinol cord blood (18 of 20 [placebo group], 10 of 10 [subtherapeutic allopurinol and/or oxypurinol group], and 7 of 15 [therapeutic allopurinol and/or oxypurinol group]; $P < .05$). Seven placebo and 2 subtherapeutic allopurinol and/or oxypurinol cord samples were hemolytic and NPBI could therefore not be determined reliably. Figure 4 shows the box-whisker plots of the free radical markers.

DISCUSSION

To our knowledge, this pilot study is the first to show that allopurinol administered to pregnant women in labor with fetal distress crosses the placenta in a similar way compared with pregnant women with healthy fetuses on the brink of delivery.[18] Fetal allopurinol and/or oxypurinol concentrations, however, were lower in arterial cord blood in newborns who suffered from fetal hypoxia compared with healthy counterparts as reported in the study by Boda et al.[18]

Furthermore, we found that, if therapeutic allopurinol and/or oxypurinol concentrations could be established in the fetus, S-100B concentrations were substantially lower in arterial cord blood, suggesting less brain damage after fetal hypoxia.

However, several questions remain, which are partly due to the study set-up and the fact that the number of patients included in this pilot study was small. First, it was not possible to reach therapeutic allopurinol and/or oxypurinol levels in all neonates. This may have been because of the brief period between treatment and actual birth of the infant, which prevented optimal passage of allopurinol from the mother to the fetus. This problem is difficult to solve because the obstetrician will prefer a quick delivery of the hypoxic fetus. In clinical practice, however, this problem may be less pressing, because additional delay is more likely to occur in a study set-up because of the time taken for explanation of the study and obtaining informed consent. Moreover, it is possible that a brief delay of delivery is acceptable if a clear improvement of the fetal condition can be verified by recovery of the fetal heart rate or fetal scalp blood samples, gaining some time to achieve optimal fetal allopurinol concentrations. Another reason for the sometimes subtherapeutic fetal allopurinol and/or oxypurinol levels may be that the dosage used in this pilot study, which was based on placental passage of (orally administered) allopurinol in healthy pregnant women in labor,[18] was too low because of hindered placental passage as a result of suboptimal placental function or intermittent umbilical cord occlusion. Given the absence of any adverse effect of neonatal or maternal allopurinol treatment in all human studies performed to date,[9,13,16,18,19] a higher dosage will be considered in a larger study that is currently being planned. Although oral administration may be an option in labor complicated by fetal hypoxia, we could not detect allopurinol or oxypurinol in arterial cord blood of 2 additional cases of fetal hypoxia in which we treated the mother with 600 mg of allopurinol orally (unpublished observation).

Second, only a short-term chemical end point with respect to brain damage and neurologic outcome was evaluated in the present study: the S-100B concentration in cord blood. S-100, and in particular its brain specific B-subunit, S-100B in urine and/or blood, have been reported to be indicators of cerebral injury,[23–24] severity of post–hypoxic-ischemic encephalopathy,[33] and neurodevelopmental outcome[34] in preterm and term infants who suffered from birth asphyxia. A very recent article reports on the relationship between S-100B in umbilical blood at birth and acidosis and pathologic heart rate patterns during labor.[25] Moreover, a recent study by Berger et al[35] in infants with traumatic brain injury showed a strong relationship between S-100B and outcome. However, long-term follow-up with a detailed assessment of neurodevelopmental outcome of the children treated antenatally with allopurinol or a placebo will be necessary to prove a neuroprotective effect of antenatal allopurinol

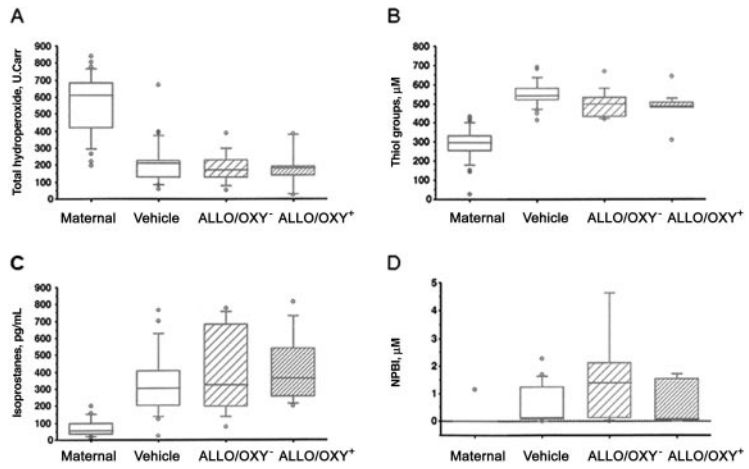

FIGURE 4

Maternal and arterial cord concentrations of the placebo-treated (placebo), therapeutic allopurinol and/or oxypurinol (ALLO/OXY$^+$), and subtherapeutic allopurinol and/or oxypurinol (ALLO/OXY$^-$) groups of total hydroperoxide (U.Carr) (A), thiol groups (μM) (B), isoprostanes (pg/mL) (C), and NPBI (μM) (D), during birth shown as box-whisker plots.

treatment. For this type of study, we estimate that a total of ~200 to 240 cases are needed to assess the effect of maternal allopurinol treatment on neurodevelopmental outcome after fetal hypoxia. This rather large number of inclusions is necessary, because the outcome of the mature fetus suffering from fetal hypoxia can be very variable as shown in earlier studies[36–38] and by the rather normal 5-minute Apgar scores and umbilical artery pH values of the 3 groups in the present study.

A final comment should be made concerning the free radical marker concentrations measured in our study. Although there was an indication that free radical production was less in the therapeutic allopurinol and/or oxypurinol fetuses, no significance was detected. However, the fact that significantly fewer cord blood samples had detectable NPBI in the therapeutic allopurinol and/or oxypurinol group may indicate that allopurinol did reduce free radical production. Furthermore, we are also unable to explain the lack of difference between groups with respect to plasma uric acid in the arterial cord blood. Assuming a substantial inhibition of xanthine oxidase activity in the therapeutic allopurinol group, one would expect lower uric acid concentrations. We postulate that the time between allopurinol administration and measurement of uric acid concentration may have been too short to assess differences in between groups. Furthermore, for xanthine oxidase to generate uric acid, reperfusion and reoxygenation are required. This was not the case in the cord blood that was used for uric acid determination.

Despite the considerations and uncertainties mentioned above, which may in part be related to the small sample size of the present pilot study, the preliminary results including the significantly lower S-100B concentrations in the therapeutic allopurinol group make it worthwhile to design a larger trial. In this trial the most important end point should be a clear marker of long-term neurodevelopmental outcome.

CONCLUSIONS

This pilot study shows that allopurinol crosses the placenta during fetal hypoxia. Given the considerably lower umbilical cord allopurinol and oxypurinol concentrations in the present study compared with concentrations measured after oral administration of an equivalent allopurinol dose to mothers in uncomplicated labor,[18] it is conceivable to assume that a higher allopurinol dosage should be administered during fetal hypoxia to achieve therapeutic concentrations in the hypoxic fetus. This assumption seems realistic, because all human studies to date in which allopurinol was administered to the mother and fetus or neonate did not show any short- or long-term adverse effects. Furthermore, newborns with therapeutic allopurinol and/or oxypurinol concentrations in cord blood had lower S-100B concentrations. Therefore, a larger trial in compromised fetuses at term seems warranted.

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REFERENCES

1. Gluckman PD, Wyatt JS, Azzopardi D, et al. Selective head cooling with mild systemic hypothermia after neonatal encephalopathy: multicentre randomised trial. *Lancet.* 2005;365(9460):663–670
2. Shankaran S, Laptook AR, Ehrenkranz RA, et al. Whole-body hypothermia for neonates with hypoxic-ischemic encephalopathy. *N Engl J Med.* 2005;353(15):1574–1584
3. Jacobs S, Hunt R, Tarnow-Mordi W, Inder T, Davis P. Cooling for newborns with hypoxic ischaemic encephalopathy. *Cochrane Database Syst Rev.* 2007;(4):CD003311

4. Azzopardi D, Edwards AD. Hypothermia. *Semin Fetal Neonatal Med.* 2007;12(4):303–310
5. Barks JDE. Current controversies in hypothermic neuroprotection. *Semin Fetal Neonatal Med.* 2008;13(1):30–34
6. Palmer C, Towfighi J, Roberts RL, Heitjan DF. Allopurinol administered after inducing hypoxia-ischemia reduces brain injury in 7-day-old rats. *Pediatr Res.* 1993;33(4 pt 1):405–411
7. van der Kooij MA, Groenendaal F, Kavelaars A, Heijnen CJ, van Bel F. Neuroprotective properties and mechanisms of erythropoietine in invitro and invivo experimental models for hypoxia-ischemia. *Brain Res.* 2008;59(1):22–33
8. van den Tweel ER, van Bel F, Kavelaars A, et al. Long-term neuroprotection with 2-iminobiotin, an inhibitor of neuronal and inducible nitric oxide synthase, after cerebral hypoxia-ischemia in neonatal rats. *J Cereb Blood Flow Metab.* 2005;25(1):67–74
9. Gunes T, Ozturk MA, Koklu E, Kose K, Gunes I. Effect of allopurinol supplementation on nitric oxide levels in asphyxiated newborns. *Pediatr Neurol.* 2007;36(1):17–24
10. Gunn AJ, Bennet L, Gunning MI, Gluckman PD, Gunn TR. Cerebral hypothermia is not neuroprotective when started after postischemic seizures in fetal sheep. *Pediatr Res.* 1999;46(3):274–280
11. McCord JM. Oxygen-derived free radicals in postischemic tissue injury. *N Engl J Med.* 1985;312(3):159–163
12. Saugstad OD, Aasen AO. Plasma hypoxanthine concentrations in pigs: a prognostic aid in hypoxia. *Eur Surg Res.* 1980;12(2):123–129
13. Van Bel F, Shadid M, Moison RM, et al. Effect of allopurinol on postasphyxial free radical formation, cerebral hemodynamics, and electrical brain activity. *Pediatrics.* 1998;101(2):185–193
14. Moorhouse PC, Grootveld M, Halliwell B, Quinlan JG, Gutteridge JM. Allopurinol and oxypurinol are hydroxyl radical scavengers. *FEBS Lett.* 1987;213(1):23–28
15. Ko KM, Godin DV. Inhibition of transition metal ion-catalysed ascorbate oxidation and lipid peroxidation by allopurinol and oxypurinol. *Biochem Pharmacol.* 1990;40(4):803–809
16. Benders MJ, Bos AF, Rademaker CM, et al. Early postnatal allopurinol does not improve short term outcome after severe birth asphyxia. *Arch Dis Child Fetal Neonatal Ed.* 2006;91(3):F163–F165
17. Chaudhari T, McGuire W. Allopurinol for preventing mortality and morbidity in newborn infants with suspected hypoxic-ischaemic encephalopathy. *Cochrane Database Syst Rev.* 2008;(2):CD006817
18. Boda D, Nemeth I, Kiss P, Orvos H. Treatment of mothers with allopurinol to produce therapeutic blood levels in newborns. *Prenat Neonatal Med.* 1999;4(2):130–134
19. Clancy RR, McGaurn SA, Goin JE, et al. Allopurinol neurocardiac protection trial in infants undergoing heart surgery using deep hypothermic circulatory arrest. *Pediatrics.* 2001;108(1):61–70
20. Anonymous. FIGO guidelines for the use of fetal monitoring. *Int J Gynaecol Obstet.* 1987;25(2):159–167
21. Wiberg-Itzel E, Lipponer C, Norman M, et al. Determination of pH or lactate in fetal scalp sampling in management of intrapartum fetal distress: randomised controlled multicenter trial. *BMJ.* 2008;336(7656):1284–1287
22. Groenendaal F, Lindemans C, Uiterwaal CS, de Vries LS. Early arterial lactate and prediction of outcome in preterm neonates admitted to a neonatal intensive care unit. *Biol Neonate.* 2003;83(3):171–176
23. Maschmann J, Heinemann MK, Ziemer G, Speer CP. Evaluation of protein S-100 serum concentrations in healthy newborns and seven newborns with perinatal acidosis. *Acta Paediatr.* 2000;89(5):553–555
24. Gazzolo D, Florio P, Ciotti S, et al. S100B protein in urine of preterm newborns with ominous outcome. *Pediatr Res.* 2005;58(6):1170–1174
25. Stuart A, Edvinsson L, Kallen K, Olofsson P, Hellsten C, mer-Wahlin I. Fetal electrocardiographic monitoring during labor in relation to cord blood levels of the brain-injury marker protein S-100. *J Perinat Med.* 2008;36(2):136–141
26. Puig JG, Casas EA, Ramos TH, Michan AA, Mateos FA. Plasma oxypurinol concentration in a patient with allopurinol hypersensitivity. *J Rheumatol.* 1989;16(6):842–844
27. van Kesteren C, Benders MJ, Groenendaal F, van Bel F, Ververs FF, Rademaker CM. Population pharmacokinetics of allopurinol in full-term neonates with perinatal asphyxia. *Ther Drug Monit.* 2006;28(3):339–344
28. Milne GL, Sanchez SC, Musiek ES, Morrow JD. Quantification of F2-isoprostanes as a biomarker of oxidative stress. *Nat Protoc.* 2007;2(1):221–226
29. Paffetti P, Perrone S, Longini M, et al. Nonprotein-bound iron detection in small samples of biological fluids and tissues. *Biol Trace Elem Res.* 2006;112(3):221–232
30. Masaoka N, Nakajima Y, Hayakawa Y, et al. Transplacental effects of allopurinol on suppression of

oxygen free radical production in chronically instrumented fetal lamb brains during intermittent umbilical cord occlusion. *J Matern Fetal Neonatal Med.* 2005;18(1):1–7

31. Graham S, Day RO, Wong H, et al. Pharmacodynamics of oxypurinol after administration of allopurinol to healthy subjects. *Br J Clin Pharmacol.* 1996;41(4):299–304
32. Nemeth I, Boda D. A method for determination of the inhibition of xanthine oxidase activity in plasma during allopurinol treatment. *Eur J Clin Pharmacol.* 1978;13(6):423–426
33. Nagdyman N, Kömen W, Ko HK, Müller C, Obladen M. Early biochemical indicators of hypoxic-ischemic encephalopathy after birth asphyxia. *Pediatr Res.* 2001;49(4):502–506
34. Nagdyman N, Grimmer I, Scholz T, Müller C, Obladen M. Predictive value of brain-specific proteins in serum for neurodevelopmental outcome after birth asphyxia. *Pediatr Res.* 2003;54(2):270–275
35. Berger RP, Beers SR, Richichi R, Wiesman D, Adelson PD. Serum biomarker concentrations and outcome after pediatric traumatic brain injury. *J Neurotrauma.* 2007;24(12):1793–1801
36. Robertson CM, Finer NN. Long-term follow-up of term neonates with perinatal asphyxia. *Clin Perinatol.* 1993;20(2):483–500
37. Groenendaal F, Fetter WP, Baerts W. A study of the sequelae of perinatal hypoxia and convulsions in full-term newborn infants. *Ned Tijdschr Geneeskd.* 1990;134(25):1223–1227
38. Low JA, Galbraith RS, Muir DW, Killen HL, Pater EA, Karchmar EJ. The relationship between perinatal hypoxia and newborn encephalopathy. *Am J Obstet Gynecol.* 1985;152(3):256–260

Maternal Allopurinol During Fetal Hypoxia Lowers Cord Blood Levels of the Brain Injury Marker S-100B

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